Immunohistochemical Study of a Monoclonal Antibody 115D8 against Human Milk-Fat Globule Membrane (Mam-6) in Some Histological Types of Breast Cancer.

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原　　著

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Summary

An antigen, MAM-6, in human milk-fat globule membranes, was detected with a monoclonal antibody 115D8, in paraffin-embedded sections of 148 cases including human breast cancers and other breast diseases with immunoperoxidase technique. There were some differences on the staining portion in the cells among the different types of breast cancers, although we could not find any differences in intensity of reaction with 115D8 among the different histochemical types of breast cancers. MAM-6 was mainly localized in the apical portion of the cells or the cytoplasms of the papillo-tubular carcinoma. The antigen was chiefly localized in the margin of the cells or cytoplasms of the solid- tubular carcinoma and scirrhous carcinoma. Normal mammary glands, sweat glands and sebaceous glands were reacted with 115D8 but epidermis, esophagus, stomach, small intestines, large intestines, pancreas, liver, lung, kidney,urinary bladder, thyroid, adrenal glands, heart, striated and smooth muscles, spleen, lymphnodes and brain were not reacted with 115D8 in this study. Although the antibody was not sufficient for differential diagnosis among the types of breast cancer, it may be useful to detect the breast cancer.

Introduction

In recent years, several monoclonal antibodies which react with neoplastic cells have been introduced in mammary carcinomas[1-20]. Human milk-fat globule (HMFG) membranes were reported to be one of such potential markers for breast cancers[11,19]. HILKENS et al. have reported the production of panel of monoclonal antibodies directed against differentiation antigens of the mammary gland[11]. 115D8 is one of these antibodies and reactive with MAM-6, which is a sugarprotein with more molecular weight than 400 k dalton, and an epithelial membrane marker present at the

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apical side of epithelial cells lining normal ductal and alveolar structures in the breast[11].

The monoclonal antibody, 115D8, can detect a carbohydrate antigen 15–3 (CA15–3) with together use of a monoclonal antibody DF 3 which was directed against a membrane fraction of human breast cancer[12]. CA15–3 was found abundantly in the sera of patients with cancers of breast, ovary, prostate, uterine cervix, pancreas, lung or stomach[8,16].

In this study, we performed immunohistochemical study of 115D8 in 94 breast cancers, 54 benign breast diseases, 7 other cancers and some normal organs and compared distributions of MAM–6.

Materials and Methods

A total of 148 cases which were undergone biopsy or mastectomy for breast diseases were investigated. Table 1 shows number of cases of each type of diseases: 94 cases were breast cancers, 38 cases mastopathies, 9 cases intraductal papillomas, 4 cases mastits, and 3 cases fibroadenomas. Tissues were fixed with 10 percent neutral buffered formalin and routinely embedded in paraffin and sectioned. Regular hematoxylin and eosin (H & E) sections were used for morphologic evaluation. We used the classification of the Japanese society for the study of breast cancer.

For immunohistologic study of MAM–6, the indirect immunoperoxidase method (Avidin-Biotin (ABC) method) was performed. After deparafinization and hydration in the usual manner, the sections were washed in phosphate-buffered saline (PBS) at pH 7.6 for 5 minutes. Endogenous peroxidase activity was blocked with hydrogen peroxide (0.5 percent in methylalcohol) for 5 minutes, and they were rinced with PBS. Then they were preincubated with normal swine serum (1 ml/100 ml PBS) for 20 minutes for blocking non-specific reaction and incubated with the monoclonal antibody 115D8 diluted 1 : 500 (Centocor, Inc., Malvern, PA, U.S.A.) at room temperature for 20 minutes. After rincing, they were incubated with biotinylated anti-mouse antibody (Vector Laboratories Inc.) for 20 minutes, rinced, and incubated with Avidin DH/biotinylated peroxidase for 20 minutes at room temparature (VECTASTAIN ABC kit). Color reactions were developed with 0.4 mg/ml of 3 amino, 9-ethylcarbazole and one drop of 1% hydrogen peroxide in 0.05 M sodium acetate buffer at pH 4.9. The sections were counterstained with hematoxylin and mounted in geratin.

For the assesment of the reaction, we classificated three types by the difference of the staining portion in the cells: A, apical side of the cell, microvilli (Fig. 1); B, margin of the cell, plasma membrane (Fig. 2); C, cytoplasm (Fig. 3). Moreover, we attempted to grade the intensity of the reaction microscopically, as follows: (+++), more than 2/3 areas occupied by the positive cells in the cancer area; (++), from 1/3 to 2/3 areas occupied by the positive cells in the cancer; (+), less than 1/3 areas; (−), the tissues having no reacted cells with 115D8.

We used a staining index to compare the reaction of each histopathological type. The staining index was calculated as follows:

$$\text{Staining index} = \frac{3 \times \text{No. of}(+++) + 2 \times \text{No. of}(++) + \text{No. of}(+)}{\text{Total No. of cases}}$$

Results

All of 94 cases of breast cancers showed positive reaction, though some differences in the intensi-

Table 1 Histopathological Diagnosis of 148 Breast Specimens.

Diagnosis	No. of cases	
Breast cancer	94	
Non-Invasive		(6)
Intraductal papillary carcinoma	6	
Invasive		(88)
Papillotubular carcinoma	27	
Solid-tubular carcinoma	30	
Scirrhous carcinoma	26	
Mucinous carcinoma	2	
Medullary carcinoma	3	
Benign lesions	54	
Intraductal papilloma	9	
Mastopathy	38	
Fibroadenoma	3	
Mastitis	4	
Total	148	

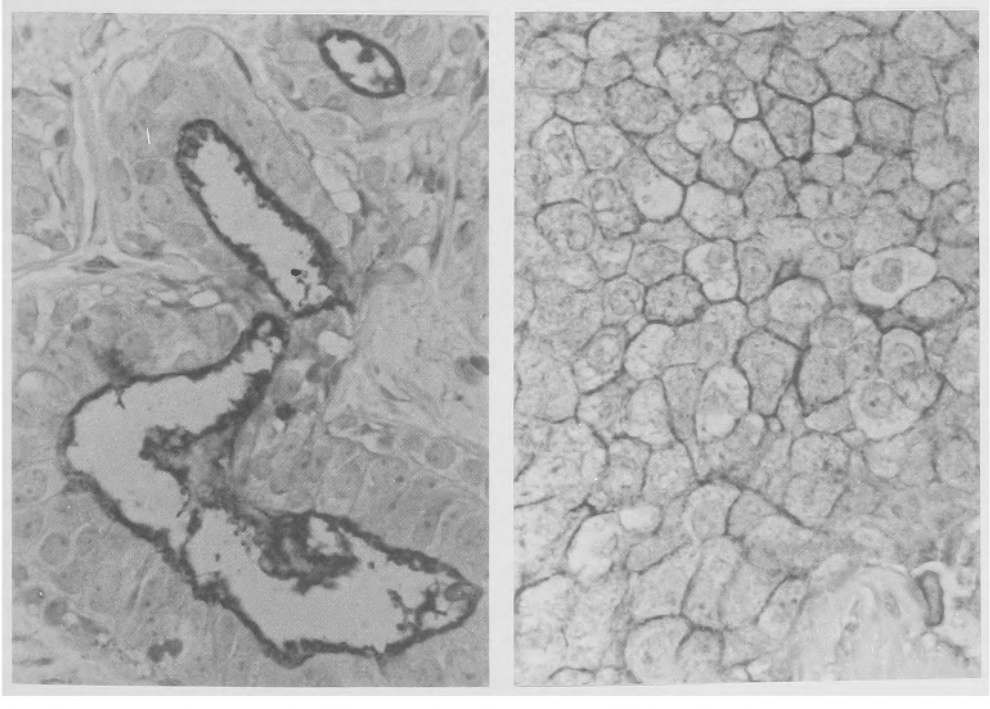

Fig. 1 Normal mammary glands with positive reaction at the luminal surfaces (A type). 115D8 immunoperoxidase reaction with hematoxylin. ×780.

Fig. 2 Solid-tubular carcinoma with positive reaction at the margins of the cells (B type). 115D8 immunoperoxidase reaction with hematoxylin. ×780.

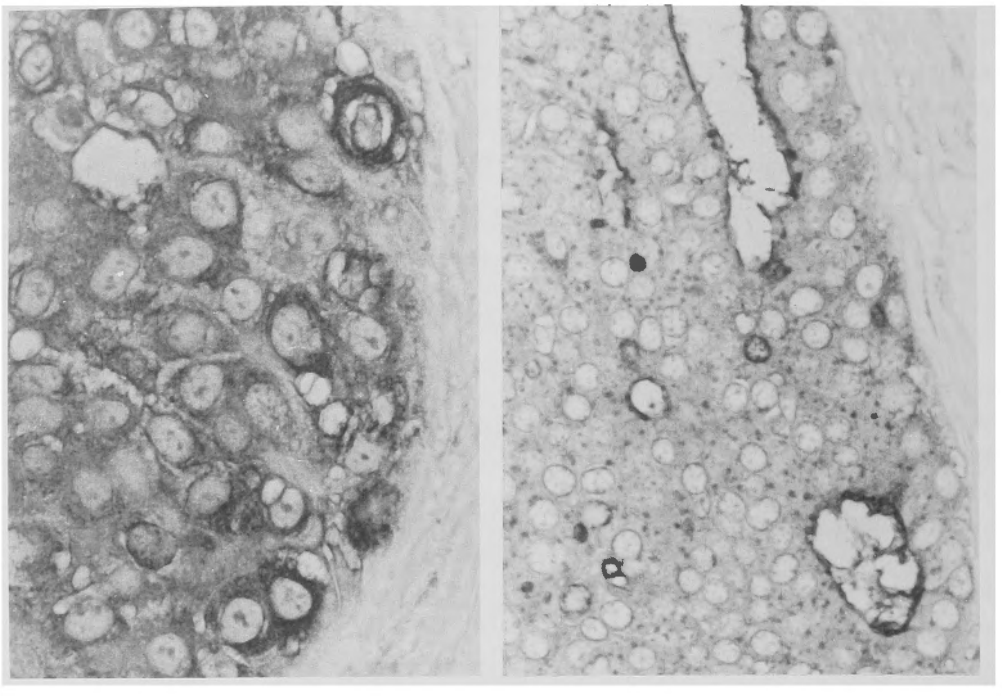

Fig. 3 Papillary carcinoma with positive reaction at the whole cytoplasms (C type). 115D8 immunoperoxidase reaction with hematoxylin. ×780.

Fig. 4 Papillary carcinoma with positive reaction at the luminal surfaces and the cytoplasms (A+C type). 115D8 immunoperoxidase reaction with hematoxylin. ×390.

Table 2 Correlation between Three Histological Types of the Breast Cancer and the Staining Site and Intensity of Immunohistochemistry for 115D8.

Histological type of breast cancer	No.	A*			B*			C*			Staining Index**		
		+++	++	+	+++	++	+	+++	++	+	A	B	C
Papillotubular Carcinoma	27	6	7	12	0	0	7	7	5	10	1.63	0.26	1.52
Solid-tubular Carcinoma	30	5	3	5	9	6	6	9	7	5	0.87	1.50	1.53
Scirrhous Carcinoma	26	2	1	8	8	6	5	9	6	7	0.62	1.56	1.77

* Staining site A; Apical side of the cell or microvilli, B; margin of the cell or plasma membrane, C; cytoplasm.

** Staining Index $= \frac{3 \times \text{No. of}(+++) + 2 \times \text{No. of}(++) + \text{No. of}(+)}{\text{No. of cases}}$

ty of the reaction were present. In non-infiltrating papillary carcinomas, the reaction was mainly localized at apical portion of the cells (A), and at the margin of the cells and/or cytoplasms in some areas. In the papillotubular carcinoma cells were mainly stained at apical portion of the cells (A), cytoplasms (C) or both (A+C type, Fig. 4). However, the margin of the cells (B, Fig. 2), cytoplasms (C), or both (B+C type, Fig. 3) were observed in most of the solid-tubular carcinoma and the scirrhous carcinoma (Table 2). In the intraductal papilloma, the reaction was present also at the apical portion of the cells (A) similar to the intraductal carcinoma and we could not find the par-

ticular difference in the reactions between these two types. The reacting manner was mainly A and C type in mucinous carcinoma and B and C type in medullary carcinoma, though only a few cases of both types of carcinomas were examined in this study.

The acini and ducts of normal mammary glands, normal sweat glands, normal sebaceous glands were positively reacted. The sweat glands were stained at the margin of the cells (B type). The acini and ducts of the mammary glands, sebaceous glands were stained mainly at the apical portion of the cells (A type) and rarely in the cytoplasms (C type). The myoepithelium in the mammary glands was negatively reacted. Benign mammary diseases, mastopathy, mastitis, and epithelial components in fibroadenoma were positively reacted with similar manner to those of normal mammary glands and ducts.

Cancers in other organs except ovarian adenocarcinoma were all negative for MAM-6 reacted with 115D8 such as a gastric cancer, a colon cancer, a hepatocellular carcinoma, and three lung carcinomas (a small cell carcinoma, an adenocarcinoma and an epidermoid carcinoma). Only ovarian adenocarcinoma had a few positive areas. Most of normal organs, such as heart, bronchus, lung, esophagus, stomach, small intestine, colon, liver, pancreas, gall bladder, kidney, urinary bladder, thyroid, adrenal gland, striated muscle, smooth muscle, spleen, lymphnode, mesotherium, fat tissue and epidermis were negative for MAM-6 reacted with 115D8.

Discussion

115D8 which is one of the monoclonal antibody to MAM-6 has positive reaction with all 94 cases of breast cancers, though all of cancer cells could not be stained. There were some differences of reacting feature among the different types of breast cancers. The surface cells forming the glandular structure were mostly reacted at the apical portion, but the cells that do not face to the cavities were reacted in the cytoplasms or at the margins of the cells. It is supposed that reacting pattern is varied by the absence of glandular structure. In the papillotubular carcinoma, it was mostly shown A+C type and in the solid-tubular carcinoma and scirrhous carcinoma it was mostly shown B+C type for this reason. However, no differences were observed in the intensity of the reaction among the types of breast cancers.

It was reported that the epithelium of many normal organs such as mammary gland, salivary gland, sweat gland, endometrium, follicular epithelium of the ovary, testis, esophagus, stomach, pancreas, lung, kidney, urinary bladder, pituitary gland, thyroid, Hassal corpsucles in the thymus and activated mesothelial cells were reacted with 115D8 in high concentration (1/100) immunohistologically[11]. We have preliminary tested immunohistochemical reactions at the several concentrations including the same concentration as HILKENS et al. did [11] and the similar results were obtained in the examination at the same concentration. Then we used a lower concentration of 115D8 to get the better correlation between the specificity and the intensity of the staining. With more diluted antibody many organs such as esophagus, stomach, small intestine, colon, lung, bronchus, liver, pancreas, gall bladder, kidney, urinary bladder, thyroid, adrenal gland, epidermis were nagative for immunohistochemical staining, while the sweat gland, sebaceous gland and mammary gland and duct were still positive. This may be due to low sensitivity of attenuant antibody to those tissues.

It was reported that the adenocarcinoma, the squamous cell carcinoma and the small cell carcinoma of the lung, the colorectal carcinoma, the gastric carcinoma, the cervical carcinoma, the

ovarian carcinoma, the endometrial carcinoma, the bladder carcinoma, the prostate carcinoma and the sweat gland carcinoma were positively reacted (12/12, 16/21, 4/4, 18/19, 12/14, 7/9, 53/53, 9/9, 3/3, 1/4, and 1/3)[11]. But we could not detect MAM-6 in our limited number of cancers in several organs with this low concentration of the antibody though the ovarian adenocarcinoma was still positive.

This antibody 115D8 is thought to be useful to diagnose the breast cancer and its glandular structure with property diluted concentration immunohistochemically.

References

1) Arklie J, Taylor-Papadimitriou J, Bodmer W, et al: Differentiation antigens expressed by epithelial cells in the lactating breast are also detectable in breast cancers. Int J Cancer **28**: 23–29, 1982.
2) Burchell J, Wang D, Taylor-Papadimitriou J: Detection of the tumor-associated antigens recognized by the monoclonal antibodies HMFG-1 and 2 in serum from patients with breast cancer. Int J Cancer **34**: 763–768, 1984.
3) Colcher D, Horan Hand P, Nuti M, et al: A spectrum of monoclonal antibodies reactive with human mammary tumor cells. Proc Natl Acad Sci **78**: 3199–3203, 1981.
4) Colcher D, Horan Hand P, Nuti M, et al: Differential binding to human mammary and non-mammary tumors of monoclonal antibodies reactive with carcinoembryonic antigen. Cancer Invst **1**: 127–138, 1983.
5) Epenetos AA, Canti G, Taylor-Papadimitriou J, et al: Use of two epithelium-specific monoclonal antibodies for diagnosis of malignancy in serous effusions. Lancet **2**: 1004–1006, 1982.
6) Epenetos AA, Britton KE, Mather J, et al: Targeting of iodine-123-labelled tumor-associated monoclonal antibodies to ovarian, breast and gastrointestinal tumors. Lancet **2**: 1000–1004, 1982.
7) Foster CS, Edwards PAW, Dinsdale EA, et al: Monoclonal antibodies to the human mammary gland, I. Distribution of determinants in non-neoplastic mammary and extra mammary tissues. Virchow Arch B **394**: 279–293, 1982.
8) Gang Y, Adachi I, Ohkura H, et al: CA15-3 is present as a novel tumor maker in the sera of patients with breast cancer and other malignancies. Jpn J Cancer Chemother **12**: 2379–2386, 1985.
9) Gatter KC, Abdulaziz Z, Beverley P, et al: Use of monoclonal antibodies for the histopathological diagnosis of human malignancy. J Clin Pathol **35**: 1253–1267, 1982.
10) Hand P, Nuti M, Colcher D, et al: Difinition of antigenic heterogeneity and modulation among human mammary carcinoma cell populations using monoclonal antibodies to tumor-associated antigens. Cancer Res **43**: 728–735, 1983.
11) Hilkens J, Buijs F, Hilgers J, et al: Monoclonal antibodies against human milkfat globule membranes detecting differentiation antigens of the mammary gland and its tumors. Int J Cancer **34**: 197–206, 1984.
12) Kufe D, Inghirami G, Abe M, et al: Differential reactivity of a novel monoclonal antibodies (DF3) with human malignant versus benign breast tumors. Hybridoma **3**: 223–231, 1984.
13) Menard S, Tagliabue E, Canevari S, et al: Generation of monoclonal antibodies reacting with normal and cancer cells of human breast. Cencer Res **43**: 1295–1300, 1983.
14) Nuti M, Teramoto YA, Mariani-Costantini R, et al: A monoclonal antibody (B72.3) defines patterns of distribution of a novel tumor associated antigen in human mammary carcinoma cell population. Int J Cancer **29**: 539–545, 1982.
15) Papsidero LD, Croghan GA, O'connel MJ: Monoclonal antibodies (F36/22 and M7/105) to human breast carcinoma. Cancer Res **43**: 1741–1747, 1983.
16) Pons-Anicet DMF, Krebs BP, Mira R: Value of CA 15-3 in the follow-up of breast cancer patients. Br J Cancer **55**: 567–569, 1987.
17) Rasmussen BB, Hilkens S, Hilgers J, et al: Monoclonal antibodies applied to primary human breast carcinoma: relationship to menopausal status, lymphnode status and steroid hormone receptor content. Breast Cancer Res Treatment **2**: 401–405, 1982.
18) Sloane JP Ormerod MG: Distribution of epithelial membrane antigen in normal and neoplastic tissues and its value in diagnostic tumor pathology. Cancer **47**: 1786–1795, 1981.
19) Taylor-Papadimitriou J, Peterson JA, Arklie J, et al: Monoclonal antibodies to epiterium-specific components of

the human milkfat globule membrane: production and reaction with cells in culture. Int J Cancer **28**: 17–21, 1981.
20) Thompson CH, Jones SL, Whitehead RH, et al: A human breast tissues-associated antigen detected by a monoclonal antibody. J Natl Cancer Inst **70**: 409–419, 1983.
21) To A, Coleman DV, Dearnley DP, et al: Use of antisera to epithelial membrane antigens for the cytodiagnosis of malignancy in serous effusions. J Clin Pathol **34**: 1326–1332, 1981.

和文抄録

乳癌における人乳汁脂肪球膜に対するモノクロナール抗体 115D8 の免疫組織学的検討

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乳癌マーカーとして臨床的に用いられている CA15-3 は人乳汁脂肪球膜の糖蛋白 (MAM-6) に対するモノクロナール抗体 115D8 と乳癌肝転移巣に対するモノクロナール抗体 DF3 により認識されているが，今回 115D8 について乳癌，乳腺良性腫瘍，その他の正常組織に対し ABC 法により免疫組織学的に，その細胞内局在を検索した．乳癌では全例が反応し組織型により，その細胞内局在に相違が認められ乳頭腺管癌では大部分が癌巣尖端部分，細胞質が染色され，一方充実腺管癌，硬癌では主に癌巣辺縁ないしは細胞質が染色された．

良性乳腺腫瘍，正常乳腺，汗腺，皮脂腺では腺上皮および管上皮が反応したが，表皮，食道，胃，小腸，大腸，膵，肝，脾，肺，腎，膀胱，甲状腺，副腎，心，脳，横紋筋，平滑筋，リンパ節などは染色されなかった．115D8 は乳癌の組織型別診断には不充分であるが，乳癌そのものの診断には有用であることが示唆された．